Fig. 1

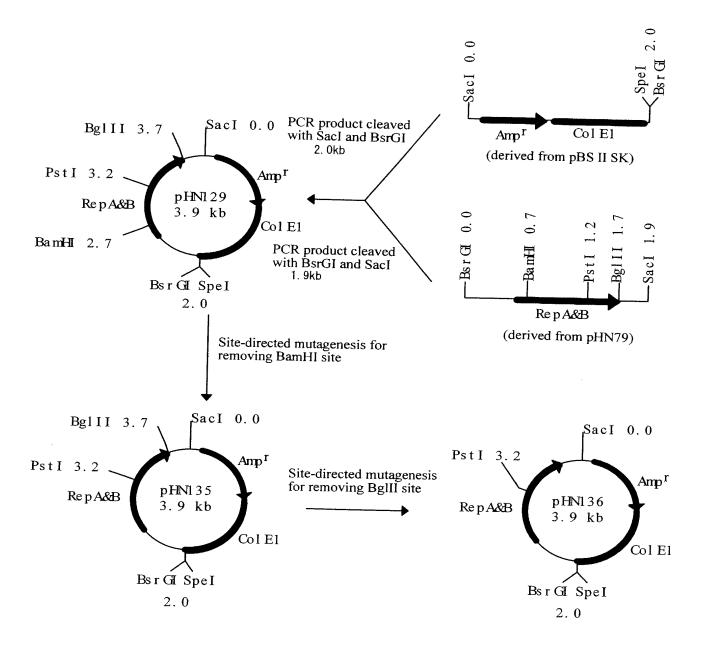


Fig. 2

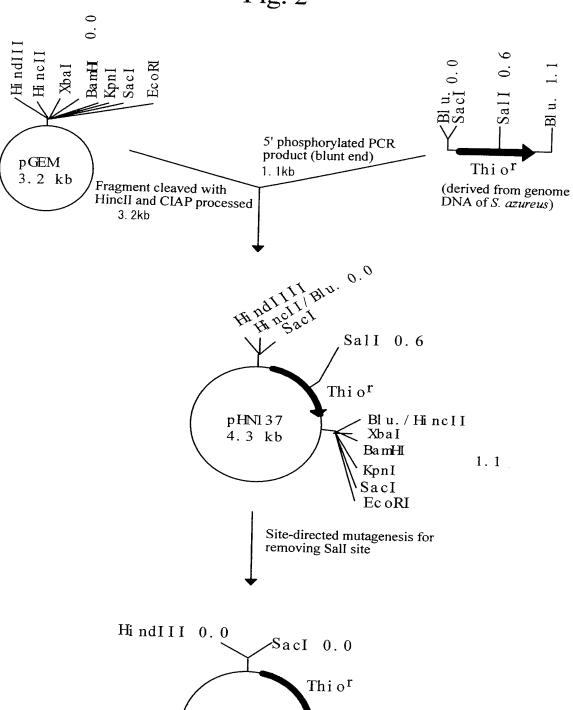


Fig. 3

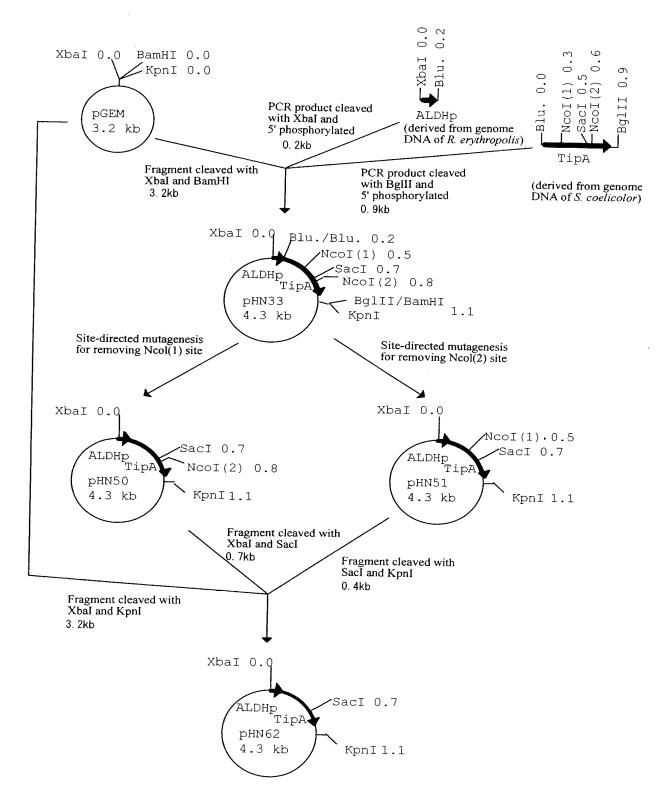


Fig. 4

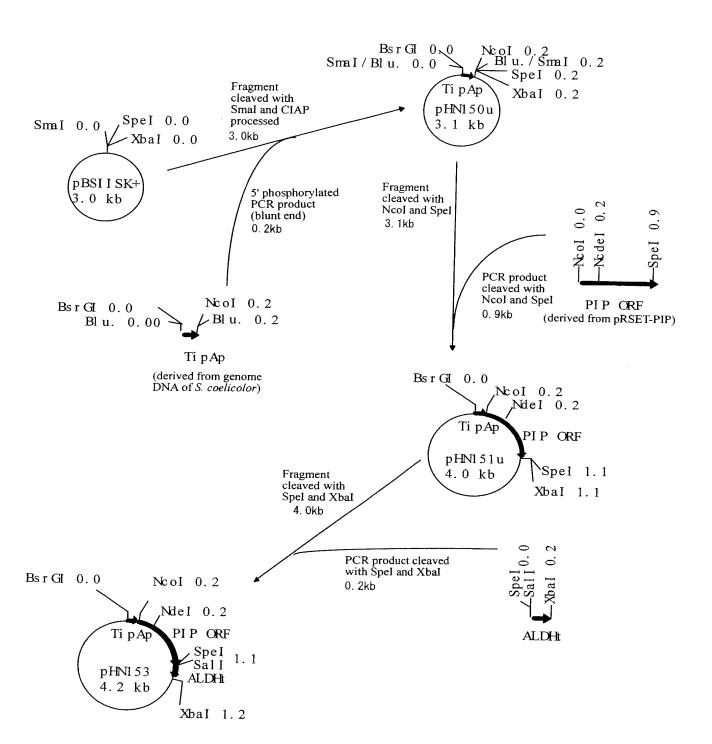
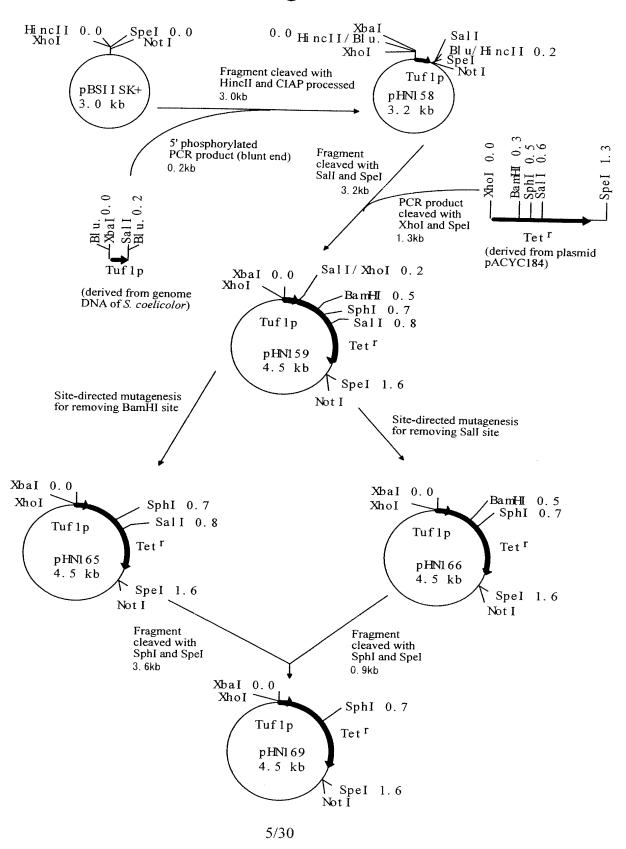
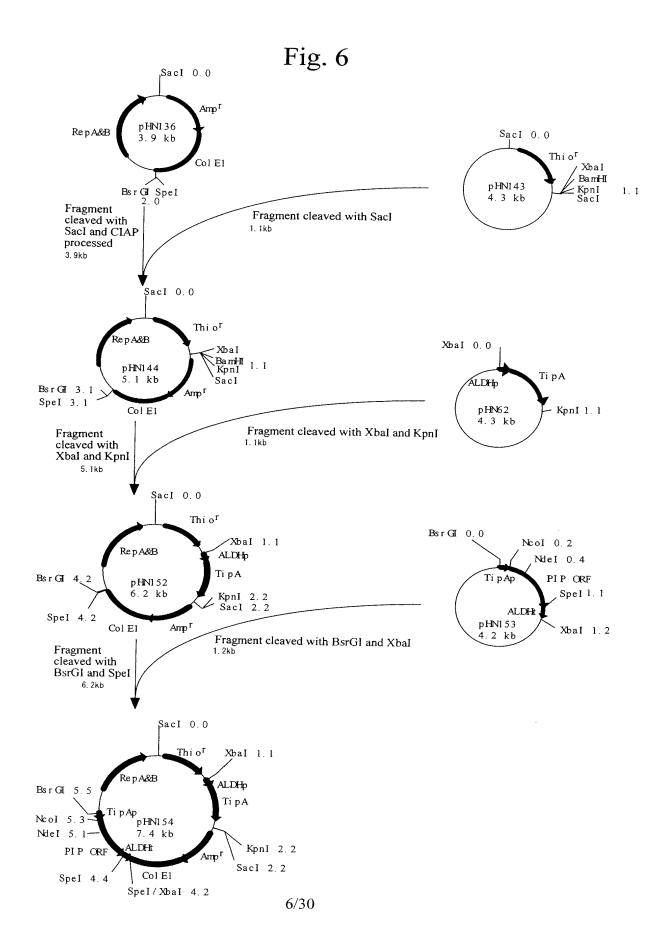


Fig. 5





KpnI 3.8 Sacl 0.0 Thi o^r pHNI 71 9.0 kb Ampr Ti pA- LGI 0p Rep A&B Col El Ndel 6,6 PIP ORF Ncol 6.8 Bsr Gl 7.0 SpeI 6. 0 PCR product cleaved with BsrGl and Ncol (derived from plasmid pHN170) Fragment cleaved with BsrGI and NcoI 8. 8kb NcoI 0.2 SpeI 1.6 Ti pA-LG10p Tet ^r BsrG 0.0 pHN169 4.5 kb Tuf 1p XbaI 0.0 Xbal/Spel 2.7 Fragment cleaved with XbaI and Spel 1.6kb Xbal 1.1 KpnI 2.2 Sacl 2.2 Tet ^r ALDH XbaI 1.1 ALDH Ti pA Thi or Amp Sacl 0.0 SacI 0.0 Thi or Spel/Xbal 4.2 pHNI 70 9. 0 kb Amp Ti pAp pHNI 54 7.4 kb RepA&B Col El Col E1 RepA&B VLDH ALDH ТірАр Fragment cleaved with Xbal and CIAP processed Spel 4,4 PIP ORF PIP ORF Bsr G 5.5 Reol 5.37 Ndel 5.1-Bsr Gl 7.0 NcoI 6.8 NdeI 6.6-Spel 6.0 7. 4kb

Xbal 1.1

Tet ^r

ALDH Ti pA

Sacl 3.8

Fig. 6 (continued)

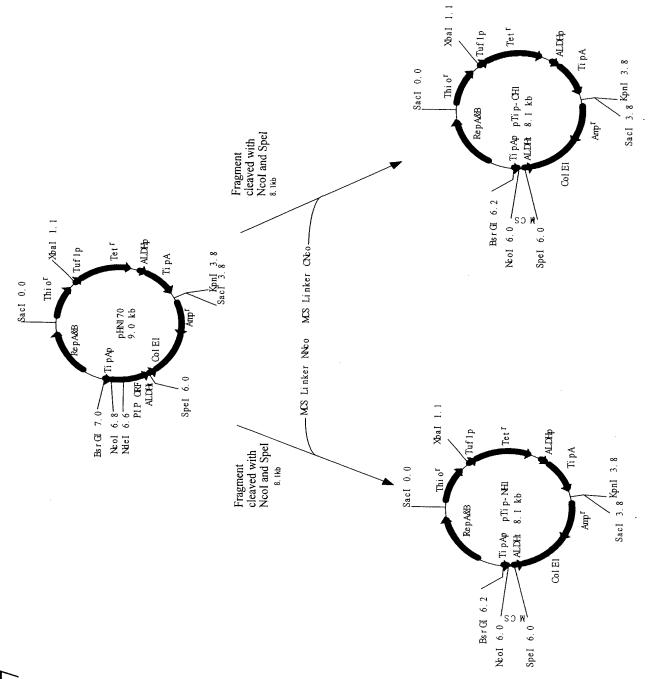


Fig. ′

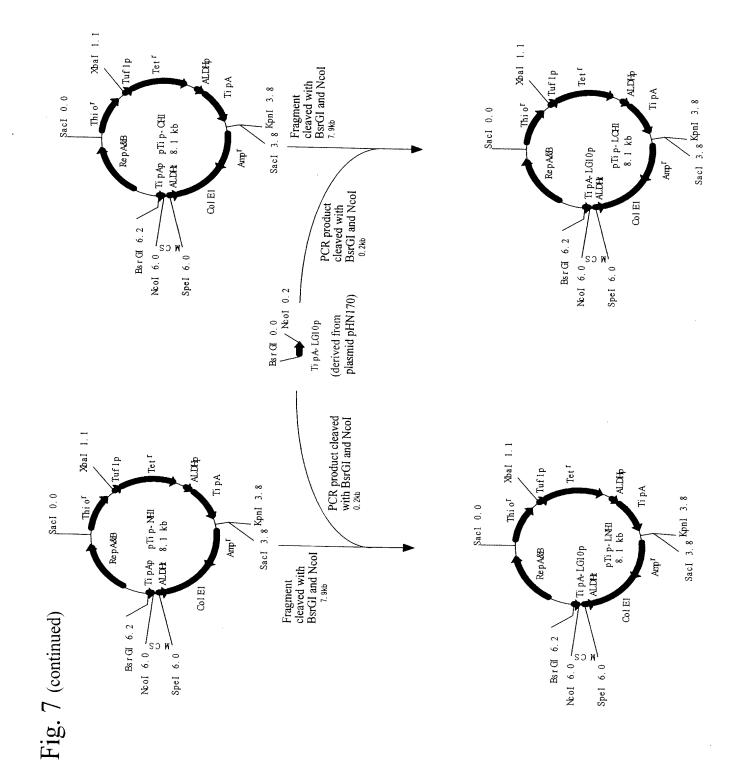
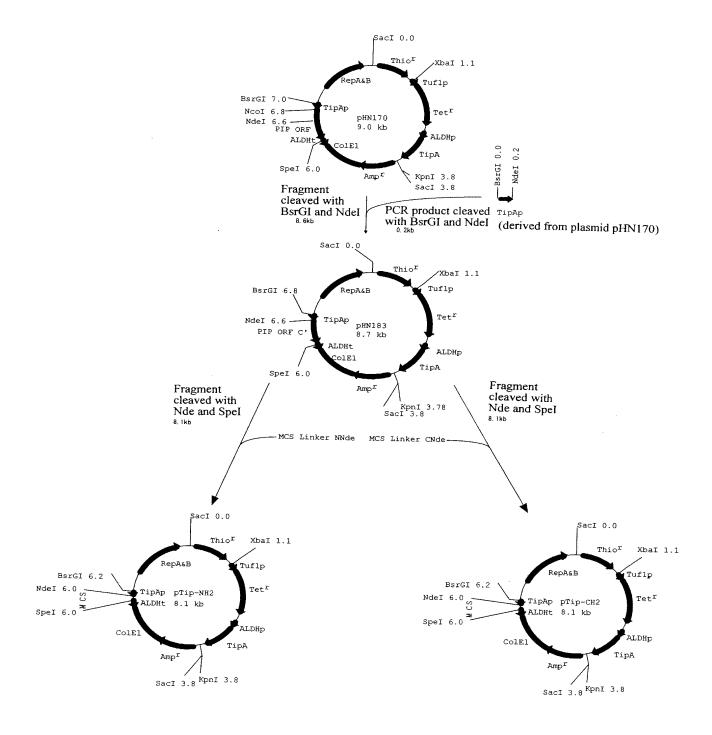


Fig. 8



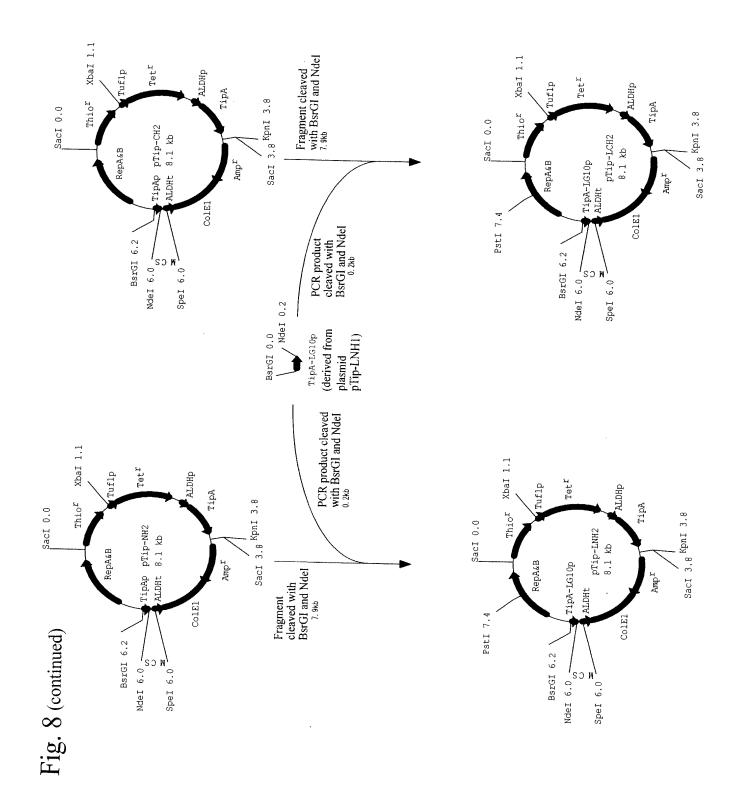
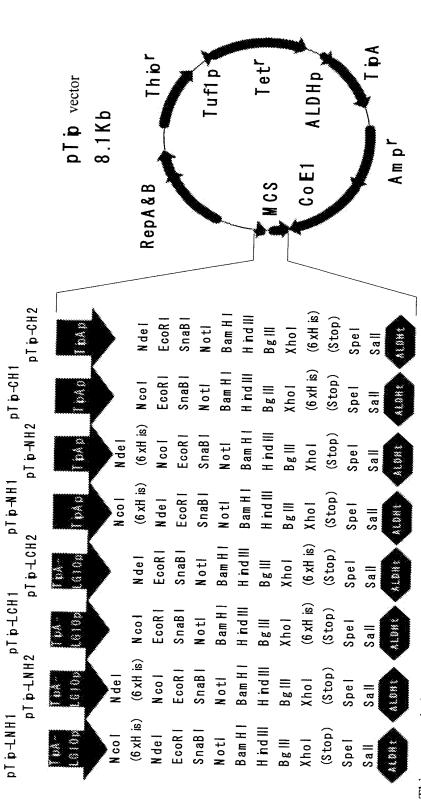


Fig. 9a



Thiostrepton induction system

Thio r = confers thiostrepton resistance to *R.erythropolis*

ALD Hp = promoter which constitutively produces TipA protein

T ip A = encodes a Tip A protein
T ip A p = Tip A promoter

TipA LG10p = improved TipA promoter ALDHt = transcription termination sequence

Regions necessary for the autonomous replication of a plasmid 6 o E1 = for *E. coli*

RepA&B = for R.erythropolis

Antibiotic resistance marker

Iufl p-I et = transformation marker for R. erythropolis

Am p^r = transformation marker for E. coli

GGC ACG CGG CGC ACG GCG TGG CAC GCG GAA CGT CCG GGC <u>Ittg CAG</u> CTC -35

ACG TCA CGT GAG GAG GCG TGG ACG GQG TCA GAG AAG GGA GCG GCQ ATG TO TTA ACT TTA AGA AGG AGA TAT ACC

GGC CAC CAT CAC CAT ATG GGA ATT CTA CGT AGC GGC CGC GGA TCC GIY His His His His His His Met GIY IIe Leu Arg Ser GIY Arg GIY Ser

AAG CTT AGA TCT CGA GGA TGA ACT AGT CGA CCC ACC GGC ACC CGT GAG CCC

CTC GCT GCG GGT GCG AGG GAC TGC AAC ACG CGA AAC CTG CAC AAA

CAC ACG GAG GTT GGA ATG AGC GCC ACG GAC ACA CCC GAT ACC GGC GCC GTT

CCA CCC CGG TTG GTG ACC ACC GCT GGG GCT GAC CTG CTA CGC CGC CTC

Bsr61 GTG TAC ATA TCG AGG CGG GCT CCC ACG GCC GGC CGG GCT GAG GGA GCC GAC

GGC ACG CGG CGG CTC ACG GCG TGG CAC GCG GAA CGT CCG GGC ITTG CAG CTC

ACG TCA CGT GAG GAG GCG TGG ACG GQG TCA GAG AAG GGA GCG GCQ ATG RBS

GTC TAG AAA TAA TTT TGT TTA ACT TTA AGA AGG AGA TAT ACC

GGA ATT CTA CGT AGC GGC CGC GGA TCC AAG CTT AGA TCT CGA GGA CAT CAC GIY AFF GIY AFF GIY AFF GIY Ser LYS Leu Arg Ser Aff GIY His His

CAT CAC CAT CAC TGA ACT AGT CGA CCC ACC GGC ACC CGT GAG CCC CTC GCT

GOG GGT GCC GGT GCG AGG GAC TGC AAC ACG CGA AAC CTG CAC AAA CAC ACG

GAG GTT GGA ATG AGC GCC ACG GAC ACA CCC GAT ACC GGC GCC GTT CCA CCC

CGG TTG GTG ACC ACC GCT GGG GCG GCT GAC CTG CTA CGC CGC CTC AGC GGG

ACT CTA GT

Bsr61 GTG TAC ATA TCG AGG CGG GCT CCC ACG GCC CGG GCT GAG GGA GCC GAC

GGC ACG CGG CGG CTC ACG GCG TGG CAC GCG GAA CGT CCG GGC TTG CAC CTC

ACG TCA CGT GAG GAG GCA GCG TBG ACG GCB TCA GAG AAG GGA GCG CAT ATG RBS

G TCT AGA AAT AAT TIT GIT TAA CTI TAA GAA GGA GAT ATA CAT

GGC CAT CAC CAT CAC CAT CAC GCC ATG GGA ATT CTA CGT AGC GGC CGC GGA GIY His His His His Ala Met GIY IIe Leu Arg Ser GIY Arg GIY

BanHI HindIII BE/III All Spell Spell

CCC CTC GCT GCG GGT GCG AGG GAC TGC AAC ACG CGA AAC CTG CAC

AAA CAC ACG GAG GTT GGA ATG AGC GCC ACG GAC ACA CCC GAT ACC GGC GCC

GTT CCA CCC CGG TTG GTG ACC ACC GCT GGG GCG GCT GAC CTG CTA CGC CGC

CTC AGC GGG ACT CTA GT

BS/GI GTG TAC ATA TCG AGG CGG GCT CCC ACG GCC CGG GCT GAG GGA GCC GAC GGC ACG CGG CGC CTC ACG GCG TGG CAC GCG GAA CGT CCG GGC <u>[TG_CAC]</u> CTC

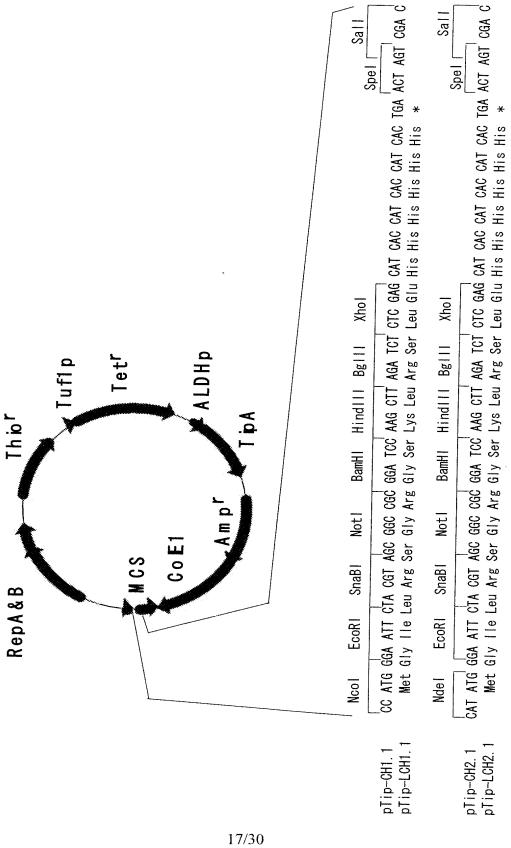
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GAG GTT GGA ATG AGC GCC ACG GAC ACA CCC GAT ACC GGC GCC GTT CCA CCC

CGG TTG GTG ACC ACC GCT GGG GCG GCT GAC CTG CTA CGC CGC CTC AGC GGG





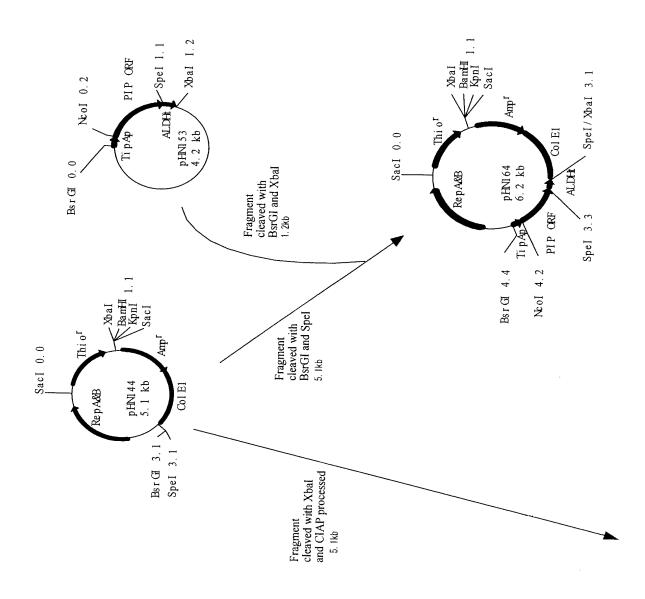
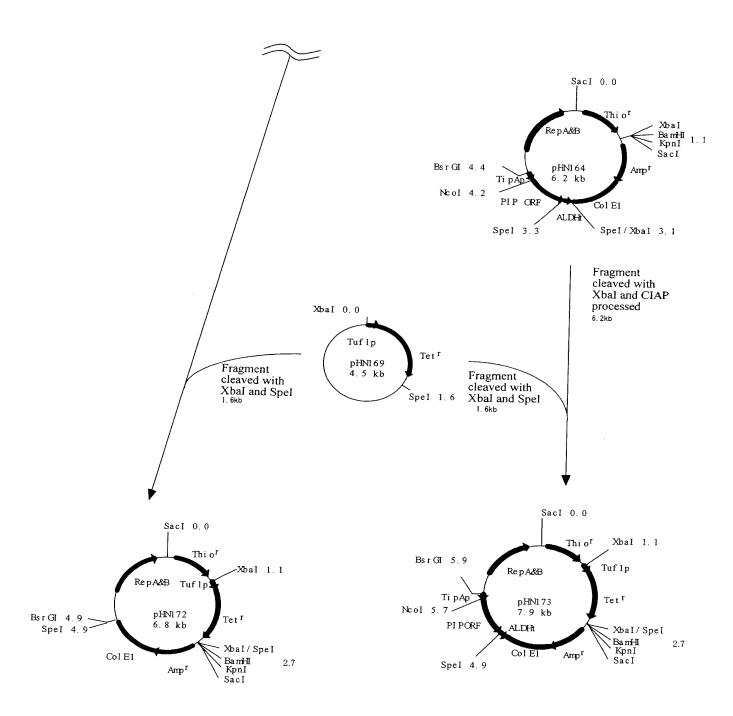
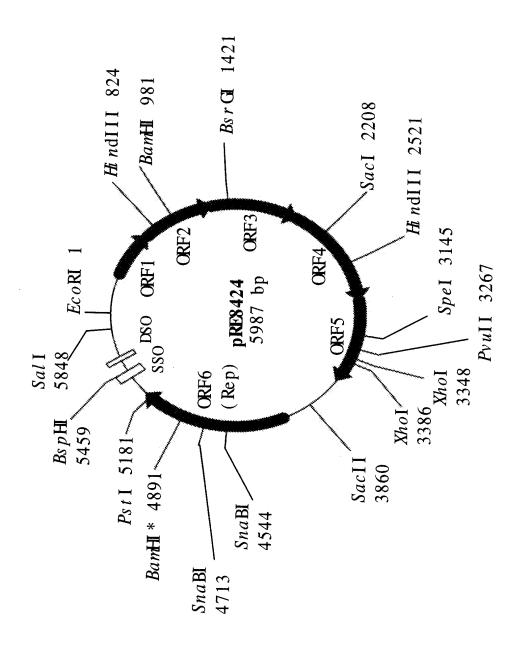


Fig. 11 (continued)



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Fig. 13



Motif 111	l a XYXXKXq X	LAANLTKI AS I GNYWSKMOT MATYLAKGNS LI BYLTKNQD LABYI AKTQD LABYI AKTQD
		53 67 54 80 69
Motif II	g XXg XXr a Xe Xi Xg XXn GwHXHXh Xl X	GCDGYVRAVEI THGK- NGWHVHVHALL GLVGYVRANEI THGK- HGWHVHSHVLI VEHIYSDYEVTDS WA- NGWFLHRNM.L GYI GWRAAEVTRSKKNGYHPHLNLLV GYVGM RATEVTVGQI NGWHPHI HAI V GYVGM RATEVTVGQI NGWHPHI HAI V
		33 33 34 77 59
Motif I	Xvt XTXRH	MTMTMRH MLTLTQRH MFVGTVRH LVTFTARH LVTFTARH LVTFTARH LVTFTARH
		26 27 27 27 27 27
Motif IV	GLXXCGXXWKCPXC	GLRSCGKGW CPCC GLHTCGS VWACP VC GLVRCGRI W.CPVC GLMRCGRI W.CPVC GLMRCGRI W.CPVC ** ** ** **
		68 138 38 20 20
	Consensus	pRE8424 pAP1 pBL1 pJV1 pIJ101 pSN22

C-terminal motif

We y EXa XXgr Ra i XWr g1 r	276 WREFEFGSMGRRAI AWSRGLR	WKEYEKASFGRRALTWSKGLR	WREYEVGSKNLRS-SWSRGAK	WAQYEEALAGRRAI EWTRGLR	WHEYERATRGRRAI EWTRYLR	288 WEYERATKGRRAI EWTRYLR	· · · * · * * · · *
	276	365	250	352	288	288	
Consensus	pRE8424	pAP1	pBL1	pJ VI	pIJ101	pSN22	

```
CGAKKOCKANGCG- [GA- COCCG]----KAGCTGGGGGAG-
                                 GALATIACAA-GIGA-AGAGEIICTaaggaaccgca--
                                                                  GAGGGAAAA-CCGA-ACACCTT--CGGAAAGAA--
                                                                                   GACCIONAMACETOTCOCOCOCOMO - COCAMAGAAA--
                                                  CTGGGANANAGGGA--10GGGT---|NGGTAAGGGGT
                   CAGGIATGC-G-GA-AAACIUT--KAGGAACAA---
                2378
1314
3375
1346
7805
p RE8 424
                                                pJ V1
pI J 1 0 1
p SN2 2
                pAP1
                               pBL1
```

DSO

Nicking site

Fig. 17

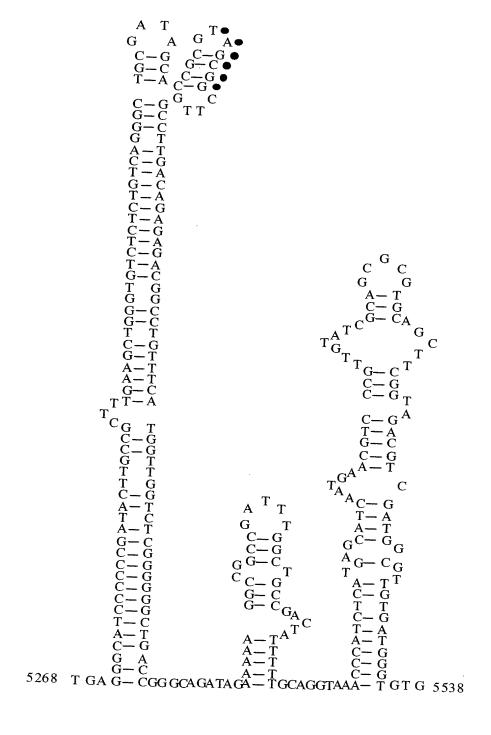
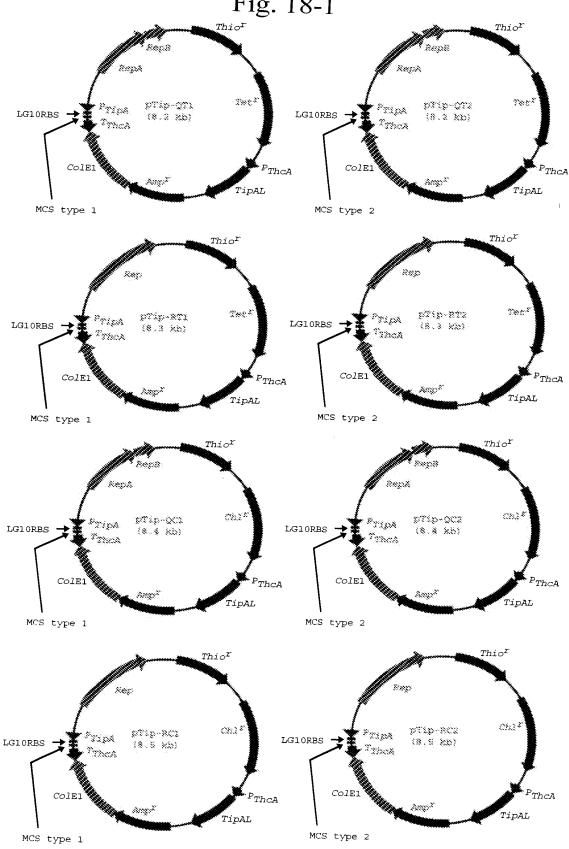


Fig. 18-1



26/30

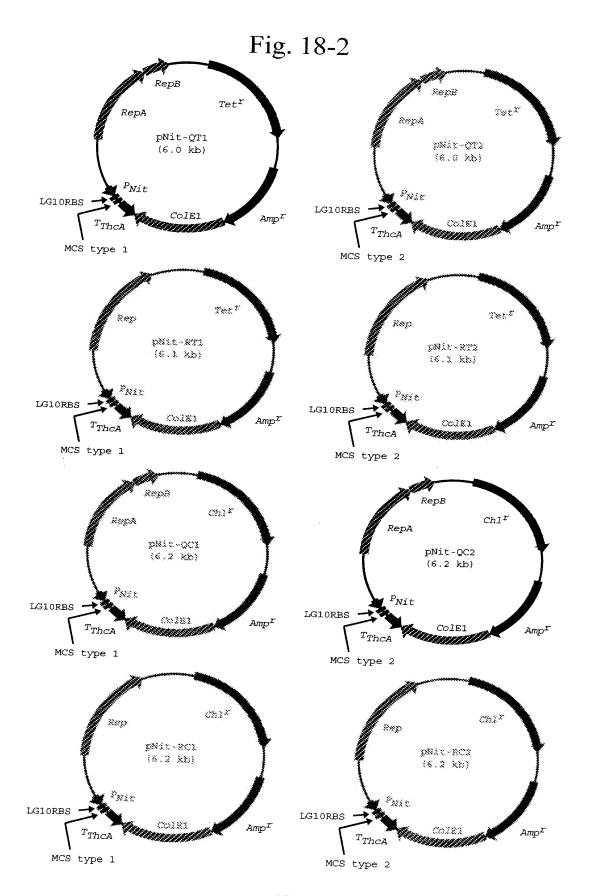


Fig. 19

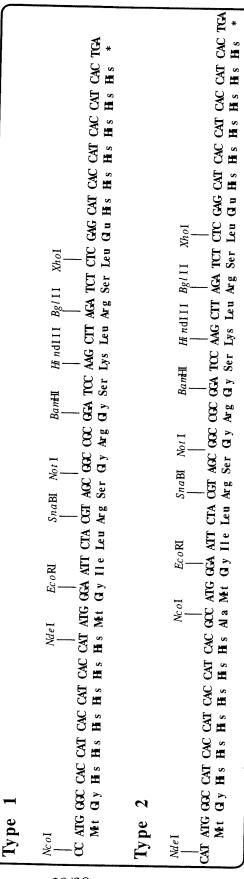
Ti pA-LG10p or Nit-LG10p

38.7 G

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TIG CAC] CTC AGG TCA OGT GAG GAG GCA GGG TCT AGA AAT AAT TIT GIT TAA CIT TAA GAA GAT AITA TA TAA T

MCS



ALDHt

Spel Sall

ÀCT AGT CGA CCC ACC GGC ACC CGT GAG CCC CTC GCT GCG GGT GCC GGT GCG AGG GAC TGC AAC ACG CGA AAC CTG CAC AAA CAC AGG GAG GTT GGA ATG AGC GCC ACG GAC ACA CCC GAT ACC GCC GTT CCA CCC GTTG GTG ACC ACC GCT GGG GCG GCT GAC CTG CTA CGC CTC AGC GGG ACT CTA GT

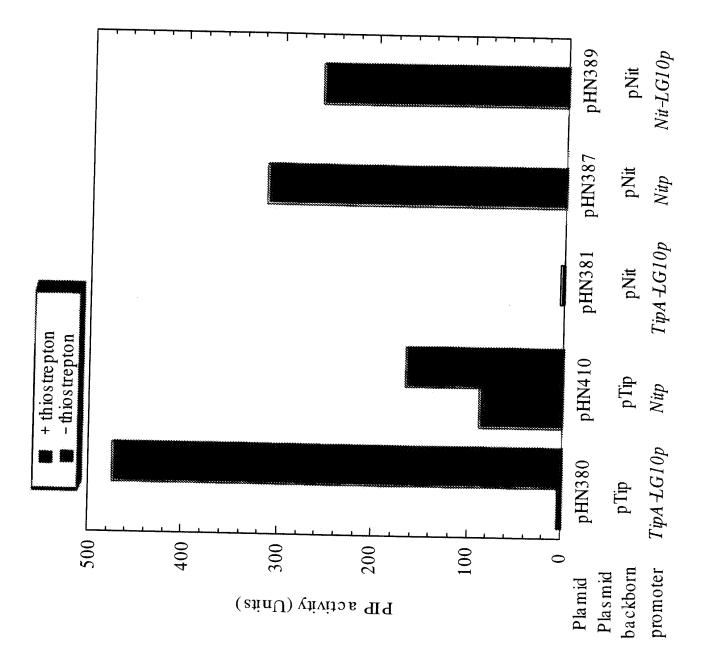


Fig. 20

